## On how and when reindeer cool their brains

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Reindeer (*Rangifer tarandus*) are protected against the Arctic cold by thick fur of prime insulative quality. The high thermal resistance of the coat makes dissipation of excess heat difficult, and hence heat-stressed reindeer resort to panting. Moderately heat-stressed resting reindeer usually display closed mouth panting, whereby blood is cooled in the richly vascularised mucosa of their nasal turbinates. This blood is drained via the dorsal nasal veins and may be routed via the facial and jugular veins for general body cooling, or via the angular oculi veins (AOV) to the cavernous sinuses for selective brain cooling (SBC), which is defined by the IUPS Thermal Commission as a brain temperature lower than carotid blood temperature. During severe heat stress and/or exercise, however, reindeer use open mouth panting (OMP), and evaporation then occurs mainly from their richly vascularised and large tongue. Under these conditions we have to assume that the efficiency of SBC is reduced, due to reduced air flow through the nose (Aas-Hansen *et al.* 2000) and the fact that the cool blood from the tongue cannot be used for SBC. SBC, which is common to artiodactyls, was originally seen as a defence mechanism against hyperthermic damages to the brain during running, but we have seen that exercise implies open mouth breathing which compromises SBC. We have surgically instrumented four adult female reindeer under isoflurane anaesthesia to allow continuous recordings of brain ($T_b$) and carotid artery ($T_a$) temperatures, and were also able to measure rectal temperature and respiratory rate, with the animal standing in a climatic chamber at ambient temperatures ranging from −20 to 40 °C. We could also occlude both AOVs, when desired, and thereby prevent SBC. The experiments were approved by the Norwegian Committee on Ethics in Animal Experimentation.

We observed that, upon occlusion of both AOVs, $T_b$ increased significantly, even in situations where $T_b$ was maintained 0.5 °C higher than $T_a$ before the occlusion. This shows that reindeer employ SBC long before $T_b < T_a$. Moreover, AOV occlusion also resulted in significantly increased respiratory rates, particularly at high ambient temperatures, at which OMP occurred most of the time. Such periods of OMP were consistently associated with a significant drop in $T_a$. Examination after humane killing revealed that the jugular veins and the common carotid arteries run in close association for a distance of 25–30 cm. We propose that counter-current heat exchange between cold venous blood from the tongue and the carotid artery blood explains the OMP-associated drops in $T_a$, and that this is sufficient to prevent a further rise in $T_b$, even when SBC is compromised due to OMP. However, OMP is costly, both in terms of energy and body water. We therefore suggest that SBC is activated to defend $T_b$ at moderate heat loads, while, as the heat load increases, the animal must resort to OMP in order to avoid general hyperthermia. $T_b$ is still defended due to counter-current heat exchange between the jugulars and the carotids, but this is expensive both in terms of energy and water, and also prevents the animal from grazing, which results in a reduced energy intake.

Aas-Hansen, O. *et al.* (2000). *Am. J. Physiol.* **279**, R1190–1195.

*All procedures accord with current national guidelines.*

## How seals may cool their brains during prolonged diving

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When seals dive for prolonged periods, their arterial oxygen tension may decrease to values as low as 2.0 kPa. The time it takes to reach such extreme values is mainly determined by the extent of a selective peripheral vasoconstriction which distributes most of a much reduced cardiac output to the brain, while the rest of the body has to rely on local stores of oxygen and anaerobic metabolism (e.g. Blix & Folkow, 1983). In this situation brain oxygen demand would be much reduced, and hence dive duration further extended, if brain temperature was reduced. We have shown that that is indeed the case (Odden *et al.* 1999). In the present study we have used six harp seals (*Pagophilus groenlandicus*) and measured brain temperature ($T_b$), carotid blood temperature ($T_c$), muscle (m. latissimus dorsi) temperature ($T_m$) and rectal temperature ($T_r$) during experimental dives of 10 min duration. The details of the methodology followed Odden *et al.* (1999), the experiments being approved by the Norwegian Committee on Ethics in Animal Experimentation. We found that heart rate always fell promptly from about 100 to 8–10 b.p.m. upon submergence and that $T_b$ fell significantly, in some cases as much as 3 °C, of which about 30 % took place during the first 5 min after the end of the dive, when the previously intense bradycardia was replaced by tachycardia (120–140 b.p.m.). $T_c$, which was about a degree lower than $T_b$ before diving, fell in parallel with $T_b$ during the dive, while $T_m$ remained fairly stable throughout the dives, with $T_r$ being reduced, probably due to local cooling from the hind flippers. Moreover, in some of the dives $T_b$ started to decline even before the seal was submerged, suggesting that the decrease in $T_b$ is not a result of passive cooling, but is under physiological control and even influenced by higher brain centres. To elucidate how this is achieved we carried out a series of anatomical studies of the vasculature of the fore flippers of three hooded seals (*Cystophora cristata*) after humane killing, by dissection and angiography using OEC Medical Systems, Inc., series 9600 X-ray equipment with Mixobar Colon (Astra Tech; 1 g ml$^{-1}$) as contrast medium. We found that the blood which enters the flippers is drained either through A-V shunts, which have been shown to be abundant in seal skin, to the large superficial saphenous veins, or by way of the capillary bed to smaller deep veins which split into a rete around the artery at the base of the flipper, where counter-current heat exchange occurs. We suggest that the animals achieve controlled cooling of the brain simply by regulating A-V shunt flow in the skin of the rather uninsulated fore flippers, whereby only those tissues which are perfused, notably the brain, are cooled. If we assume a similar $Q_{10}$-effect on cerebral metabolism in seals as in pigs, and further assume that seals can cool their brains by at least 3 °C when diving in the wild, this would result in a reduction of brain oxygen demand of 15–20 % (Busija & Leffler, 1987) and also provide neuroprotection against hypoxic injury.

Blix, A.S. & Folkow, B. (1983). *Handbook of Physiology, The Cardiovascular System* **III** (2), 917–945.

Busija, D.W. & Leffler, C.W. (1987). *Am. J. Physiol.* **253**, H869–873.

Odden, A. *et al.* (1999). *Acta Physiol. Scand.* **166**, 77–78.

*All procedures accord with current national guidelines.*

## *In vivo* gene transfer demonstrates that endogenous eNOS activity in the nucleus tractus solitarii depresses the baroreceptor reflex in conscious rats

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In a decerebrate rat model, the working heart–brainstem preparation, we demonstrated that angiotensin II activation of endothelial nitric oxide synthase (eNOS) within the nucleus tractus solitarii (NTS) depressed the baroreceptor reflex (Paton *et al.* 2001). In the present study, we have assessed whether endogenous eNOS activity in the NTS plays a role in determining the gain of the baroreceptor reflex in conscious rats.

A recombinant adenoviral vector directing expression of a dominant negative mutant form of eNOS (so-called TeNOS; Lee *et al.* 1995) was microinjected bilaterally into the NTS of rats to disable endogenous eNOS activity. We used radio-telemetry to acquire arterial pressure data and from this determined the spontaneous baroreceptor reflex gain (sBRG; see Oosting *et al.* 1997) before, and 4 weeks following, viral transfection. Both transmitter implantation and transfections were performed under anaesthesia using ketamine (60 mg $kg^{-1}$) and medetomidine (250 $\mu$g $kg^{-1}$) given I.M. and reversed with a subcutaneous injection of atipamezole (1 mg $kg^{-1}$). Changes in the sBRG induced by both exercise (wheel running, 6 m $min^{-1}$ × 10 min) and exposure to stress, caused by placing the rats in smaller cages, were assessed. Control groups received NTS microinjection of either an adenovirus that expressed enhanced green fluorescent protein (eGFP) or saline. TeNOS was detected in the NTS immunocytochemically.

There was no alteration in the basal sBRG in either control group during the 4 week observation period (eGFP, $n = 6$; saline, $n = 4$). However, over the same time course the resting sBRG increased gradually up to the third week from 1.04 ± 0.1 to 1.52 ± 0.3 ms $mmHg^{-1}$ (mean ± S.E.M., $n = 6$, Student's paired $t$ test, $P < 0.05$) in NTS transfected rats. Despite this increase in baseline sBRG, dynamic exercise decreased this to sBRG to 0.51 ± 0.07 ms $mmHg^{-1}$ in transfected rats, which was not different from the level measured prior to transfection (i.e. 0.50 ± 0.05 ms $mmHg^{-1}$; $n = 6$). Similarly, stress reduced the sBRG to the same level before and after NTS transfection (i.e. 0.76 ± 0.08 *vs.* 0.87 ± 0.20 ms $mmHg^{-1}$, n.s.). In all cases we found greater TeNOS immunoreactivity in the NTS of rats expressing the eNOS mutant protein *versus* those either expressing eGFP or microinjected with saline.

These results indicate the presence of endogenous eNOS activity in the NTS that controls the set-point level of the baroreceptor reflex gain. However, disabling eNOS activity does not alter the set-point gain reached during acute changes in baroreceptor reflex function that accompany exercise and stress.

Lee, C.M. *et al.* (1995). *J. Biol. Chem.* **270**, 27403–27406.

Oosting, J. *et al.* (1997). *J. Hypertens.* **15**, 391–399.

Paton, J.F.R. *et al.* (2001). *J. Physiol.* **531**, 445–458.

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## Novel action of some organic calcium channel blockers on frog myocardium

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Gradual loss of sarcoplasmic reticular (SR) calcium during a rest period is responsible for the rest-induced decay of force of contraction in the mammalian myocardium (Reiter, 1988). The effect of two organic calcium channel blockers (OCCB), verapamil and diltiazem, on a similar 'rest-induced decay' (RID) in the frog myocardium suggests a new mechanism of action of these drugs.

Strips of frog ventricle from isolated hearts of pithed frogs (*Rana hexadactyla*) were mounted in a temperature-controlled bath (25–28 °C) continuously perfused with a well-oxygenated solution of the following composition (mM): NaCl 110, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, Hepes 10 and glucose 10; pH 7.4. The ventricular strip was subjected to field stimulation with silver electrodes at 0.2 Hz frequency and the force of contraction recorded with a force-transducer and chart recorder. The regular rhythm was interrupted by varying rest-periods ranging from 10 to 180 s.

The amplitudes of the contractions immediately before and after each rest period were compared. In general, the amplitude of the post-rest beat was less than that of the pre-rest beat. Such 'rest-induced decay' was greater with longer rest periods. The RID was statistically significant ($P < 0.05$ with paired $t$ tests) for rest periods more than 100 s.

Diltiazem (10 $\mu$M), an OCCB, changed the pattern of 'RID' in the control solution to a 'rest-induced potentiation' (RIP) in the same preparation. The drug was nevertheless negatively inotropic, but the relative post-rest amplitude was significantly enhanced at all rest periods compared with control solution (Fig. 1, $P < 0.05$ with paired $t$ tests). A similar significant RIP was seen with verapamil (10 $\mu$M) but not with nifedipine (10 $\mu$M), also an OCCB.

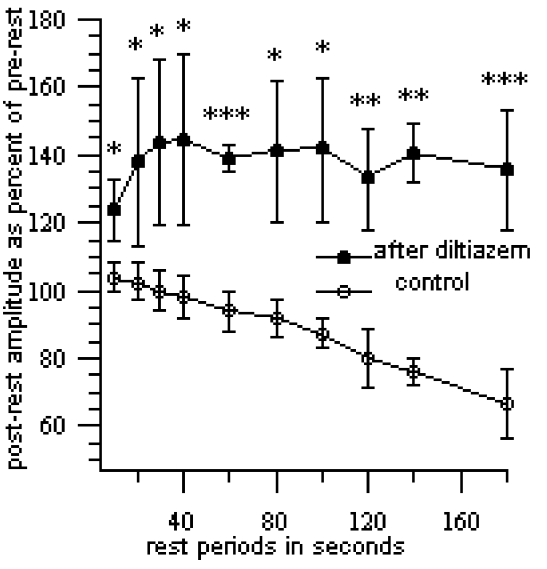


Figure 1. Effect of 10 $\mu$M diltiazem on relative post-rest contraction amplitude in strips of frog ventricle (mean ± S.D., $n = 4$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The only known mechanism of action of OCCBs, used in the treatment of hypertension and cardiac arrhythmias is

sarcolemmal calcium channel blockade, like the inorganic substance cadmium chloride. The reported RIP with verapamil and diltiazem cannot be explained by their known mechanism of action, because cadmium chloride (10 $\mu$M), though negatively inotropic, did not alter the RID of force seen in control solution.

In conclusion, the RID of force seen in frog myocardium is not due to a decreased calcium influx via the sarcolemmal calcium channels, because it is not abolished by cadmium blockade of these channels. It is therefore related to a decrease in SR calcium output following a rest period, which can be explained by a diastolic SR calcium leak as in the mammalian myocardium. We propose that the RIP in the presence of verapamil and diltiazem is due to blockade of such a leak.

Reiter, M. (1988). *Pharmacol Rev.* **40**, 189–217.

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*All procedures accord with current national and local guidelines.*

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## Effect of calcineurin inhibition and MEK1/2 inhibition upon metabolic enzyme activities in primary cultures of rat skeletal muscle

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Little is known of the signal transduction pathways that control differential gene expression in fast and slow skeletal muscle fibres. Earlier studies indicated that both the calcineurin pathway (Chin *et al.* 1998) and the ERK1/2 pathway (Murgia *et al.* 2000) can increase the percentage of slow fibres *in vivo*. Here we investigated the effect of calcineurin blockade with cyclosporin A and ERK1/2 blockade with U0126 upon the enzyme activities in primary rat skeletal muscle culture. Donor animals were killed humanely under approval of the ethical committee of the University of Copenhagen. Cells were either not treated (control) or incubated with either 0.25 $\mu$M cyclosporin A or 10 $\mu$M U0126 ($n = 4$ for each group). The enzymes measured were creatine kinase (CK), lactate dehydrogenase (LDH), hexokinase (HEX), malate dehydrogenase (MDH), citrate synthase (CS), pyruvate dehydrogenase (PDH total and percentage active form) and $\beta$-hydroxyacyl-CoA dehydrogenase (HAD). Differences as a result of treatment (control, cyclosporin A, U0126, $n = 4$ for each group) were analysed using a dependent ANOVA ($P < 0.05$ was considered significant). Both CK and LDH activity were significantly increased following cyclosporin A treatment compared with control. Activities of CK, LDH, HEX, MDH and HAD were all significantly increased above control following U0126 treatment. The percentage of PDH in its active form showed a significant decrease with U0126 treatment. These results suggest that both the calcineurin/NFAT and ERK1/2 pathway control some but not all enzymes that are expressed in a fibre type-specific manner. We conclude that it is an oversimplification to assume that a single signal transduction pathway is capable of controlling each gene that is expressed in a fibre type-specific manner. Bioinformatic approaches or microarray studies will be needed to characterize the sets of genes that are regulated by signal transduction pathways and to understand the complexity of fibre type-specific gene regulation.

Chin, E.R. *et al.* (1998). *Genes Dev.* **12**, 2499–2509.

Murgia, M. *et al.* (2000). *Nat. Cell Biol.* **2**, 142–147.

*All procedures accord with current national guidelines.*

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## Altered calcium homeostasis in heart from streptozotocin-induced diabetic rat

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An alteration in calcium ($Ca^{2+}$) mobilisation is believed to play an important physiological role in diabetes-induced cardiomyopathy (Ha *et al.* 1999). In this study we have investigated the role of $Ca^{2+}$ mobilisation in ventricular myocytes from streptozotocin (STZ)-induced diabetic rats compared with age-matched controls. STZ (60 mg kg$^{-1}$) was administered intraperitoneally (I.P.) to male Wistar rats (250–300 g). Animals were humanely killed by a blow to the head followed by cervical dislocation and ventricular myocytes were isolated by a combination of enzymatic and mechanical dispersal techniques (Howarth & Levi, 1998). $Ca^{2+}$ transients were measured at 35–37 °C in fura-2-loaded cells by fluorescence photometry. Cells were superfused with a normal Tyrode (NT) solution containing 1 mM $Ca^{2+}$. In some experiments, following a train of steady-state $Ca^{2+}$ transients, electrical stimulation was abbreviated and caffeine (20 mM) was rapidly applied to release $Ca^{2+}$ from the sarcoplasmic reticulum (SR). At 8–12 weeks after STZ treatment blood glucose levels in diabetic (mean ± S.E.M., 565.7 ± 22.9 mg dl$^{-1}$, $n = 5$) animals were significantly higher ($P < 0.01$; independent samples $t$ test) compared with controls (98.4 ± 3.9 mg dl$^{-1}$, $n = 5$). Other characteristics of diabetic animals included significantly ($P < 0.01$) reduced body weight and heart weight. Previous experiments in our laboratory have demonstrated a significant ($P < 0.05$) increase in the amplitude and the time-to-peak ($t_{pk}$) contraction in myocytes from STZ-treated compared with controls (Howarth *et al.* 2001).

The $t_{pk}$ of electrically evoked $Ca^{2+}$ transients was not significantly altered by STZ-induced diabetes. However, the time from peak to half-relaxation of the $Ca^{2+}$ transient was significantly ($P < 0.01$) longer in myocytes from diabetic (245.4 ± 8 ms, $n = 24$) compared with control (206.4 ± 9 ms, $n = 24$) rats (Fig. 1*A*). The fractional SR $Ca^{2+}$ release following rapid application of caffeine was unaltered by STZ treatment. The rate of recovery of the $Ca^{2+}$ transients, after resumption of electrical stimulation (Fig. 1*B*), was significantly ($P < 0.05$) faster in myocytes from diabetic ($3.3 \times 10^{-3} \pm 0.3 \times 10^{-3}$ ratio units s$^{-1}$, $n = 18$) compared with control ($2.2 \times 10^{-3} \pm 0.2 \times 10^{-3}$ 340/380 ratio units s$^{-1}$, $n = 9$) rats. These results indicate that STZ-induced diabetes is associated with altered $Ca^{2+}$ transport in the heart.

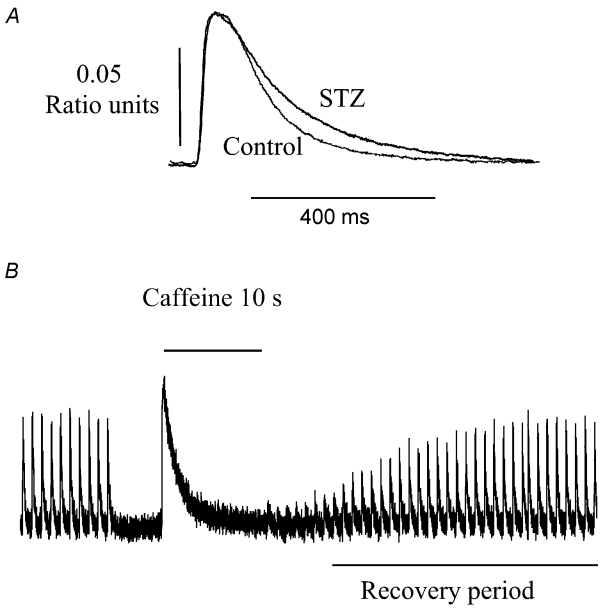


Figure 1. *A*, representative fast time-base recordings of $Ca^{2+}$ transients in electrically stimulated (1 Hz) ventricular myocytes, superfused with NT at 35–37 °C, from STZ-induced diabetic compared with control rats. *B*, typical chart recording from control heart shows the experimental protocol used to investigate SR $Ca^{2+}$ release.

Ha, T. *et al.* (1999). *Clin. Exp. Pharmacol. Physiol.* **26**, 347–353.
Howarth, F.C. & Levi, A.J (1998). *Pflügers Arch.* **435**, 687–698.
Howarth, F.C. *et al.* (2001). *Em. Med. J.* **19**, 35–41.

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*All procedures accord with current national guidelines.*

## Effect of hydrogen peroxide on cholecystokinin-induced secretory activity in mouse pancreatic acinar cells monitored by the membrane fluorescent dye FM1-43

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Stimulation of pancreatic acinar cells induces the release of digestive enzyme via exocytotic fusion of zymogen granules. Recently, optical measurements of membrane turnover have been developed that use membrane-sensitive fluorescent probes that can provide real-time measurements of secretory activity (Smith & Betz, 1996). The most extensively used of these probes is the non-permeant membrane indicator FM1-43, which is non-fluorescent in aqueous solution but becomes fluorescent when incorporated into the plasma membrane. Previous studies in pancreatic acinar cells have reported that reactive oxygen species, such as hydrogen peroxide ($H_2O_2$), are able to mobilize calcium from intracellular stores but inhibit CCK-induced calcium mobilization (Pariente *et al.* 2001). In the present study we have studied the effect of $H_2O_2$ on CCK-8-induced secretory activity by monitoring FM1-43 fluorescence using confocal microscopy. FM1-43 was excited by the 488 nm laser light line from the krypton-argon laser, and emitted light passed through a 515 nm light long-pass filter before measurement of fluorescence intensity. The optical thickness was typically 1 $\mu$m and taken through an equatorial region of an acinus. In addition, amylase release was also determined using the Phadebas blue starch method (Ceska *et al.* 1969). Cells were incubated with secretagogue for 30 min and amylase activities were expressed as a percentage of the total content of amylase at the beginning of the incubation. The donor mice were killed by rapid cervical dislocation. First of all, spontaneous exocytosis was measured by perifusion of cells with FM1-43 (1 $\mu$M) in the absence of secretagogue for 10 min. In these conditions the FM1-43 fluorescence remained constant or increased slowly over minutes but never increased by more than 5.2 ± 3.1 % (mean ± S.E.M.) with respect to the fluorescence intensity at time zero. Removal of FM1-43 rapidly reduced the cell fluorescence to a residual level. Perifusion of pancreatic acinar cells with 1 nM CCK-8 caused an increase in FM1-43 fluorescence by an additional 80.9 ± 10.7 %. This increase was observed in all 49 cells recorded, and usually consisted of a rapid initial rise, followed by a sustained plateau in fluorescence. The application of 1 mM $H_2O_2$ to acinar cells evoked a slight increase in FM1-43 fluorescence (9.3 ± 5.6 %, $n = 28$ cells), which was significantly smaller ($P < 0.05$, paired $t$ test) than that evoked by CCK-8. Additionally, simultaneous addition of CCK-8 and $H_2O_2$ clearly abolished the CCK-8-induced fluorescence increase. FM1-43 fluorescence change was only 3.1 ± 9 % ($n = 15$ cells). Similar results have been obtained in amylase experiments, where 1 mM $H_2O_2$ attenuated the secretory effect of 1 nM CCK-8 (9.2 ± 0.8 %, $n = 15$) compared with the response to CCK-8 in the absence of $H_2O_2$ (14.5 ± 0.9 %, $n = 15$). Our results show that the reactive oxygen species $H_2O_2$ inhibits the exocytotic activity induced by the secretagogue CCK. Further investigation should be carried out to clearly identify the mechanism of action of $H_2O_2$ on CCK-induced secretory activity in the exocrine pancreas.

Ceska, M. *et al.* (1969). *Clin. Chim. Acta* 437–444.
Smith, C.B. & Betz, W.J. (1996). *Nature* **380**, 531–534.
Pariente, J.A. *et al.* (2001). *J. Memb. Biol.* **179**, 27–35.

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*All procedures accord with current national guidelines.*

## Evidence for anaesthetic-mediated effects on protein expression

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Anaesthetics affect a range of cellular processes from membrane potential to free calcium concentration $[Ca]_i$ and ion channel activity. Some of these effects may play important roles in the mechanics of the production and maintenance of anaesthesia (Kress *et al.* 1987). Of equal importance are long- and short-term side effects of anaesthetics, including memory loss, disorientation and confusion. Extracellular regulated kinase 1 and 2 (ERKs 1 and 2) have been suggested to play roles in memory formation and synaptic plasticity (Cobb, 1999) and anaesthetic-regulated changes in phosphorylated ERK1 and 2 have already been shown (Woodall & Winlow, 2002). Here we examine the effects of the two general anaesthetics thiopentone, a barbiturate, and halothane, a volatile anaesthetic, on the expression of total ERK (tERK1 and 2) in PC12 cells.

PC12 cells were routinely maintained in DMEM supplemented with 10 % horse serum, 5 % fetal calf serum, 2 mM L-glutamine, 40 u $ml^{-1}$ penicillin and 100 $\mu$g $ml^{-1}$ streptomycin as previously

described (Woodall and Winlow, 2002). Cells were continuously exposed to anaesthetic for set periods of time between 20 and 140 min, run on an SDS-PAGE gel and blotted to detect total ERK1 and 2. All data were statistically analysed using ANOVA and Tukey's *post-hoc* test and are presented as means ± S.E.M.

Thiopentone (500 $\mu$M) significantly increased the amount of tERK between 20 and 100 min constant exposure. After 20 min tERK had increased from 100 ± 1.9 % of control to 232.6 ± 18.7 % ($P = 0.002$, $n = 4$). After 120 min continuous exposure there was no significant difference from control levels. Halothane (2 %) also significantly increased tERK this time between 20 and 140 min constant exposure. After 20 min halothane expression of tERK had increased from 100 ± 1.9 to 211.1 ± 4.5 % ($P = 0.001$, $n = 4$).

These results demonstrate that anaesthetics have a significant action on protein expression in this cell line. Changes in the expression of ERKs may lead to the alteration of gene regulation, cellular plasticity and cell survival responses as well as memory formation and mitogenic responses in the cell (Cobb, 1999).Therefore it is possible that these anaesthetic-induced changes in the protein may lead to effects on these CNS activities.

Cobb, M. (1999). *Prog. Biophys. Mol. Biol.* **71**, 79–150.

Kress, H.G. *et al.* (1987). *FEBS Lett.* **221**, 28–32.

Woodall, A.J. & Winlow, W. (2002). *J. Physiol.* **537. P**, 116*P*.

## Study of how physical activity and/or melatonin treatment affect the survival of female rats with induced mammary tumours

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Physical activity has received little attention as a primary strategy in cancer control, but an increasing number of epidemiological studies have addressed the question of a possible influence of physical activity on the risk of cancer (Pedersen & Hoffman-Goetz, 2000). Also, studies on the relationship between melatonin and neoplastic disease have recently become one of the most fascinating areas of pineal research. The bulk of the experimental evidence indicates the influence of melatonin on the malignant tumour formation and/or growth (Karasek & Pawlikowski, 1999).

Thus the purpose of this study was to determinate how exercise and melatonin administration can affect the survival of rats with induced mammary tumours, and their relationship with the tumours' progression. Female Sprague-Dawley rats (*Rattus norvegicus*) were administered the carcinogen DMBA (9,10-dimethyl-1,2-benzantracene). Animals were then randomized into four groups ($n = 10$) as follows: (1) DMBA rats as control group; (2) DMBA rats + exercise (swimming; 30 min/day; 5 days/week); (3) DMBA rats + melatonin ($2 \times 10^{-2}$ M; 5 days/week); (4) DMBA rats + exercise + melatonin. Rats were handled according to the guidelines of the European Community Council Directives 86/6091 EEC.

The results showed that physical activity alone induces a greater tumour growth rate (Fig. 1) but does not significantly affect survival (control group survival: 53 ± 5 days *vs.* exercise group survival: 45 ± 15 days). Intraperitoneal melatonin administration delays tumour growth and increases survival by approximately 40 % (melatonin group survival 81 ± 20 days; $P < 0.05$). The melatonin administration also counteracts the increased tumour development induced by exercise, but does not affect survival of the animals of this group (exercise + melatonin group survival: 47 ± 4 days). Student's $t$ test was used for statistical analysis of survival time, and results are expressed as means ± S.D.

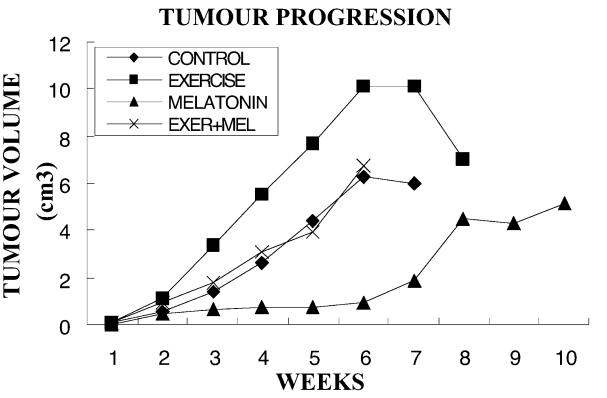


Figure 1. The effect of exercise and melatonin on the growth rate of DMBA-induced mammary tumours

In conclusion, these data suggest that melatonin could be of therapeutic help in breast cancer, slowing down the growth rate of the tumours. This is not the case for exercise, or at least in our model of physical activity.

Karasek, M. & Pawlikowski, M. (1999). *Neuroendocrinol. Lett.* **20**, 139–144.

Pedersen, B.K. & Hoffman-Goetz, L. (2000). *Physiol. Rev.* **80**, 1055–1081.

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*All procedures accord with current national guidelines.*

## Effect of moderate exercise on the functional capacity of neutrophils of sedentary individuals

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It has been suggested that moderate exercise is good for the immune system, although the effect will depend on the intensity and duration of the exercise (Nieman & Pedersen, 1999). The goal of the present work was to evaluate the effect of a single session of moderate exercise (60 % $V_{O_2,max}$) on the functional of capacity of neutrophils from sedentary individuals. Under the approval of the Ethical Committee of the University of Extremadura, six sedentary men were subjected to a 45 min session on an ergometric bicycle. The functional status of their neutrophils was evaluated before the trial, at the end of the trial, and after a further 24 h had passed. To this end, neutrophils were isolated from blood samples in a density gradient (Ficoll/Histopaque). After incubation for 60 min with *Candida albicans*, their functional capacity was evaluated by the phagocytosis index (number of candidae ingested by 100 neutrophils), phagocytosis percentage (percentage of neutrophils which have phagocytosed at least one candida) and phagocytic efficiency (mean number of candidae phagocytosed by each neutrophil that has phagocytosed at least one candida), and their

microbicidal capacity by the candidicide index (number of dead candidae) and the candidicide percentage (percentage of dead candidae per 100 neutrophils).

The results showed a significant rise ($P < 0.05$) in the phagocytosis index after exercise (153.33 ± 17.95) and 24 h later (132.00 ± 15.34) relative to the basal levels (111.67 ± 6.50). No significant differences were observed, however, in the phagocytosis percentage, although there was a significant difference ($P < 0.05$) for the phagocytic efficiency between the basal value (1.46 ± 0.11) and after the performance of the exercise (1.90 ± 0.35). With regard to the microbicidal capacity, the candidicide index rose significantly ($P < 0.05$) following the exercise (112.67 ± 13.25) and after 24 h (100.00 ± 11.59) relative to the basal value (78.00 ± 10.39). No significant differences were observed, however, in the candidicide percentage (Fig. 1).

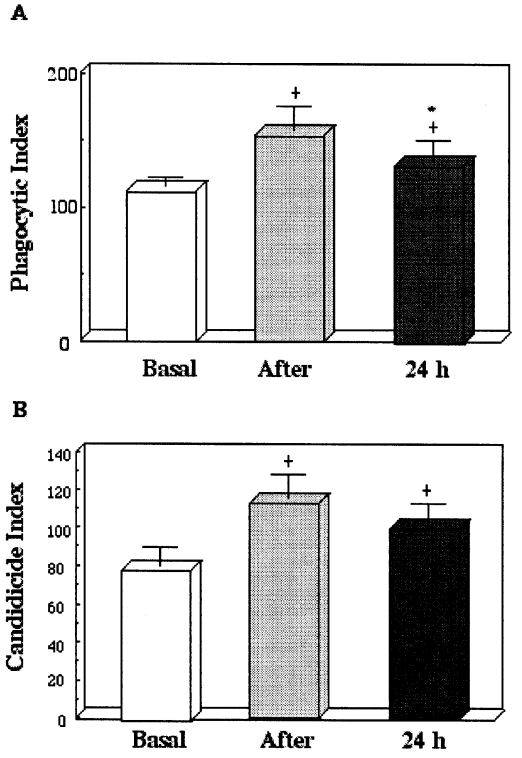


Figure 1. Phagocytosis index (*A*) and candidicide index (*B*) for neutrophils from sedentary individuals. The bar represents the mean and the standard deviation (S.D.) of 6 determinations performed in duplicate. $^{+}P < 0.05$ relative to the values obtained in the basal state (Student's unpaired *t* test). $^{*}P < 0.05$ relative to the values obtained immediately following the exercise (Student's unpaired *t* test).

A conclusion that can be drawn from the study is that, when a sedentary individual performs moderate (60 % $V_{O_2,max}$) exercise, there is a resulting protection of the host via activation of one of the groups of cells responsible for the non-specific immune response.

Nieman, D.C. & Pedersen, B.K. (1999). *Sports Med.* **27**, 73–80.

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*All procedures accord with current national guidelines.*

## Effects of aromatase inhibition on courtship behaviour, and central progesterone and androgen receptor expression in the ring dove (*Streptopelia risoria*)

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This study investigates the association between courtship behaviour, steroid receptor expression and aromatase P450 enzyme ($P450_{AROM}$) activity in the brain of the ring dove (*Streptopelia risoria*). Sexually experienced ring doves (4 males and 4 females) were injected intramuscularly with the aromatase inhibitor fadrozole (0.2 ml of 1 mg $ml^{-1}$) and paired with saline-injected controls in a cage containing a nest bowl and nesting material. Fadrozole/saline vehicle was administered for 3 days at 12 h intervals. Saline-injected control males displayed significantly (90 %) more aggressive and nest-orientated behaviours compared with fadrozole-treated males. Similarly, saline-injected control females displayed nest-oriented behaviours and were sitting on the nest by day 2, whereas fadrozole-injected females showed none of these behaviours. Following terminal anaesthesia on day 4 by intramuscular injection of pentobarbital (60 mg $ml^{-1}$), birds were perfuse-fixed with Zamboni's fixative and the brains removed for immunocytochemistry for androgen receptor (AR), progesterone receptor (PR) and $P450_{AROM}$. All data were subjected to an analysis of variance. In saline-treated birds of both sexes, nuclear AR-ir and PR-ir were observed in the nucleus preopticus, pars suprachiasmatica (POA), nucleus preopticus paraventricularis magnocellularis (PPM), nucleus preopticus medialis (POM) and nucleus preopticus medialis, pars medianis (POMm). In contrast, fadrozole treatment in both sexes resulted in the complete absence of detectable nuclear AR-ir expression, together with a significant decline in the expression of PR-ir in the POM (males 122.33 *vs.* 25.2, $P < 0.001$; females 120.89 *vs.* 29, $P < 0.001$, S.E.M.) and POMm (males 47.08 *vs.* 8.36, $P < 0.001$; females 46.33 *vs.* 18.9, $P < 0.001$, S.E.M.). A sex difference in PR-ir expression was observed in the POA (males 138.81; females 84.73, S.E.M., $P < 0.001$) and PPM (males 76.8; females 106.5, S.E.M., $P < 0.001$) of fadrozole-treated birds. In controls, AR-ir and PR-ir were co-localised in individual cells. The percentage of PR-ir cells in males containing AR-ir ranged between 46.99 ± 4.33 (POA) and 84.5 ± 2.23 (POM). The percentage of PR-ir cells in females containing AR-ir ranged between 68.42 ± 4.79 (POA) and 86.98 ± 1.93 (POM). A high density of AROM-ir cells was observed in the POM and POMm of saline-injected males, which was found to co-express PR-ir (99 %).

These results suggest that courtship behaviour may be dependent on the expression of aromatisable steroid-induced PR-ir in all preoptic regions other than the POA, and on aromatisable steroid-induced AR-ir in all preoptic regions.

*All procedures accord with current UK legislation.*

## The influence of melatonin on the phagocytic activity of rat testicular macrophages *in vitro*

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Macrophages (m$\psi$) are one of the main phagocytic cells involved in innate immunity having the ability to recognize, internalize and destroy diverse pathogens. Although the testes are considered immunologically privileged, with possible immunoinhibiting molecules (Hutson, 1994), testicular macrophages are known to interact with Leydig cells and modulate testosterone production. The pineal hormone melatonin (MLT) has numerous effects on the immune system (Maestroni, 1999) and peritoneal macrophages are known to possess MLT membrane receptors (García-Perganeda *et al.* 1999). Recently it has also been shown that diverse immune functions are affected by diabetes (Geerlings & Hoepelman, 1999).

The aim of this study was to examine whether melatonin modulates the phagocytic activity of testicular macrophages taken from control and streptozotocin (STZ)-induced diabetic rats *in vitro*.

Experiments were conducted on Wistar rats (350–400 g). Animals were killed by cervical dislocation. Testicular macrophages were washed out with PBS (30 ml/testes) and cell suspensions of $10^6$ cells ml$^{-1}$ prepared. m$\psi$ (200 $\mu$l) were incubated for 30 min at 37 °C, washed with pre-warmed PBS and 20 $\mu$l of 1 % latex beads suspension (1.1 $\mu$m diameter) added. The effect of $10^{-7}$ M, $10^{-6}$ M and $10^{-5}$ M MLT was tested on m$\psi$ from control ($n = 5$) and diabetic ($n = 5$) animals. Phagocytic index (PI) was calculated as the number of particles ingested by 100 m$\psi$. Student's paired $t$ test was used for statistical analysis and results were expressed as means ± S.E.M.

Melatonin ($10^{-7}$ M) significantly increased ($P < 0.05$) the PI in testicular m$\psi$ from control animals (PI = 68.125 ± 5 compared with PI = 46.875 ± 7 in m$\psi$ incubated with PBS). No such effect was observed on the cells from diabetic rats. Moreover, the phagocytic activity of m$\psi$ isolated from healthy rats was 20 % higher than that of m$\psi$ isolated from diabetic animals.

These results indicate that exogenous MLT may stimulate the phagocytic activity of testicular macrophages *in vitro*. Diabetes, on the other hand, may have immunosuppressive effects on these cells. The mechanism of MLT action on testicular macrophages is to be investigated.

Garcia-Perganeda, A. *et al.* (1999). *J. Neuroimmunol.* **95**, 85–94.

Geerlings, S.E. & Hoepelman, I.M. (1999). *FEMS Immunol. Medical Microbiol.* **26**, 259–265.

Hutson, J.C. (1994). *Int. Rev. Cytol.* **149**, 99–143.

Maestroni, G.J. (1999). *Adv. Exp. Med. Biol.* **460**, 395–405.

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## Influence of dietary cholesterol on the adaptation of rabbit pancreatic membranes to dietary fat

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Numerous studies show that altered dietary fat intake evokes adaptive changes in the cell membrane lipid composition. The membrane content of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA, respectively) has been shown to reflect dietary fatty acid profile (Escudero *et al.* 1998). The observed changes in the amount of saturated fatty acids (SFA) in cell membranes after elevated SFA intake suggest that the cell attempts to maintain membrane fluidity through parallel increases in MUFA levels (Burns *et al.* 1983). It has been demonstrated that inclusion of cholesterol in the diet alters membrane cholesterol content as well as its fluidity (Muriana *et al.* 1992). Moreover, dietary cholesterol seems to affect the adaptive pattern of membranes to dietary fatty acids, triggering homeostatic control mechanisms to counteract membrane rigidity (Gatto *et al.* 2001).

The aim of this study was to investigate in the rabbit pancreas whether the adaptive pattern to dietary fat is interfered with by the presence of cholesterol in the diet.

Ten New Zealand rabbits weighing 3–3.5 kg were divided into two groups and fed for 50 days with either a standard chow (Panlab, Barcelona; control group, $n = 6$) or an atherogenic diet (95 % standard chow, 3.5 % lard and 1.5 % cholesterol, w/w; atherogenic group, $n = 4$). Prior to experimentation the animals were anaesthetized with sodium pentothal (30 mg kg$^{-1}$, I.V.) and humanely killed. The pancreas was removed in order to measure total cholesterol by an enzymatic-colorimetric method and fatty acid composition of plasma membrane by gas-liquid chromatography.

Total cholesterol content, expressed as mg (g pancreas)$^{-1}$, showed significantly higher values ($P < 0.05$, one-way ANOVA) in the atherogenic group (0.18 ± 0.05; mean ± S.E.M.; $n = 4$) compared with the animals fed the control diet (0.02 ± 0.01; $n = 6$). Compared with the control group, the intake of the atherogenic diet for 50 days induced a significant ($P < 0.05$) decrease in the percentage of total SFA (44.04 ± 0.81 *vs.* 47.54 ± 1.77) and a significant ($P < 0.05$) increase of total MUFA (37.51 ± 0.27 *vs.* 34.47 ± 0.68), whereas total PUFA (18.46 ± 0.86 *vs.* 17.99 ± 1.96) and unsaturation index (1.72 ± 0.07 *vs.* 1.56 ± 0.13) were similar.

These results show that the pancreatic membranes of the atherogenic group do not reflect dietary fatty acid profile. The absence of adaptation may be associated with either (or both) of these two factors: cholesterol content and total fat content in the diet. Concerning cholesterol content, an increase in this parameter causes membranes to become more rigid and the cell tends to counteract this fact through increasing the unsaturated fatty acid content. The presence of cholesterol in the diet seems to interfere with the adaptation process of membranes by a homeostatic control to keep membrane fluidity constant.

Burns, C.P. *et al.* (1983). *Ann. Nutr. Metab.* **27**, 268–277.

Escudero, A. *et al.* (1998). *Biochim. Biophys. Acta* **1394**, 65–73.

Gatto, L.M. *et al.* (2001). *Int. J. Food Sci. Nutr.* **52**, 435–441.

Muriana, F.J. *et al.* (1992). *Biochimie* **74**, 551–556.

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## Effect of *Mormordica charantia* fruit juice on glucose and amino acid transport in L6 myotubes

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Diabetes mellitus (DM) is a metabolic disease characterised by hyperglycaemia which is associated with absolute and relative deficiencies in insulin secretion or action. Diet plays a major role in the management of DM. Prior to insulin therapy, the main form of treatment was dietary measures, including the use of traditional medicines derived mainly from plants (Platel & Srinivasan, 1997). The fruit juice of one such plant is *M. charantia* (family: Cucurbitacae) which is widely used in many developing countries as a hypoglycaemic agent to treat DM. In a previous study (Ahmed *et al.* 1999), we have shown that both the juice and its extract can regulate glucose metabolism and regenerate damaged pancreatic $\beta$-cells during DM *in vivo*. The mechanism of the hypoglycaemic action of *M. charantia in vitro* is not fully understood. In this study, we have investigated the effect of *M. charantia* juice and its extract on either $^3$H-deoxy-D-glucose or *N*-methyl-amino-$\alpha$-isobutyric acid ($^{14}$C-Me-ATB) uptake in L6 rat muscle cells cultured to the myotube stage (Hundal *et al.* 1992). The fruit was cut into small pieces and homogenised using a commercial blender. The fresh juice was centrifuged at 5000 r.p.m. and the supernatant lyophilised (fraction A). Fraction B was extracted from fraction A using chloroform and subsequently lyophilised (Day *et al.* 1990). The uptake of either $^3$H-deoxy-D-glucose or $^{14}$C-Me-ATB by L6 myotubes was performed by established methods (Hundal *et al.* 1992) in the absence and presence of different concentrations of insulin, extract A, extract B or a combination of Wortmannin (phosphatidyl inositol 3-kinase inhibitor) and the extracts over a period of 1–6 h. Total cell protein content was measured by the method of Bradford (1976). Results are expressed as pmol min$^{-1}$ (mg cell protein)$^{-1}$; $n = 6$ for each value.

Basal $^3$H-deoxy-D-glucose and $^{14}$C-Me-ATB uptakes by L6 myotubes after 1 h of incubation were (means ± S.E.M.) 32.14 ± 1.34 and 13.48 ± 1.86, respectively. Incubation of cells with 100 nM insulin for 1 h resulted in significant (ANOVA, $P < 0.05$) increases in $^3$H-deoxy-D-glucose and $^{14}$C-Me-ATB uptakes. Typically, $^3$H-deoxy-D-glucose and $^{14}$C-Me-ATB uptakes in the presence of insulin were 58.57 ± 4.49 and 29.52 ± 3.41, respectively. Incubation of L6 myotubes with three different concentrations (1, 5 and 10 $\mu$g ml$^{-1}$) of either extract A or extract B resulted in time-dependent increases in $^3$H-deoxy-D-glucose and $^{14}$C-Me-ATB uptakes, with maximal uptakes occurring after 6 h using a concentration of 5 $\mu$g ml$^{-1}$ of each extract. Typically, at a concentration of 5 $\mu$g ml$^{-1}$, extracts A and B gave uptakes of $^3$H-deoxy-D-glucose of 57.14 ± 7.85 and 52.14 ± 7.86, respectively; uptakes of $^{14}$C-Me-ATB were 33.02 ± 4.65 and 32.62 ± 2.79, respectively. Incubation of 100 nM Wortmannin with either extract A or extract B resulted in a significant ($P < 0.05$) reduction in $^3$H-deoxy-D-glucose uptake. The results suggest that *M. charantia* fruit juice and its extract are exerting their hypoglycaemic effects by stimulating glucose and amino acid uptake into skeletal muscle.

Ahmed, I. *et al.* (1999). *J. Physiol.* **520.P**, 25*P*.

Bradford, M.M. (1976). *Anal. Biochem.* **72**, 248–254.

Day, C. *et al.* (1990). *Planta Med.* **56**, 426–429.

Hundal, H.S. *et al.* (1992). *Endocrinology* **131**, 1165–1173.

Platel, K. & Srinivasan, K. (1997). *Nahrung* **41**, 68–74.

## Effects of diabetes mellitus on secretagogue-evoked secretory responses and on morphological changes in the isolated rat parotid gland

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People with diabetes mellitus (DM) suffer from a series of medical complications, including the indigestion of foodstuffs. This is due to the inability of the exocrine pancreas to secrete an adequate amount of pancreatic juice. The medical term used to describe this dysfunction is 'pancreatic insufficiency'. Like the pancreas, the parotid is also an exocrine gland that secretes mainly $\alpha$-amylase, which is required for the digestion of carbohydrates. Since the parotid is similar in structure and function to the exocrine pancreas, the aim was to investigate whether DM may also produce 'salivary insufficiency'. DM was induced in adult male Wistar rats by a single (I.P.) injection of streptozotocin (STZ) (60 mg kg$^{-1}$ body weight; Sharma *et al.* 1985). Age-matched control animals were injected with a similar volume of citrate buffer. The animals were tested for DM 4 days after STZ injection and 2 months later when they were humanely killed for the experiment. Isolated parotid glands from control and diabetic rats were used to measure amylase secretion and intracellular free calcium concentrations [$Ca^{2+}$], and to study any morphological changes in the tissues, employing established fluorimetric and light microscopic techniques (Francis *et al.* 1990; Singh *et al.* 1999).

After 2 months of STZ treatment diabetic rats weighed significantly (Student's unpaired $t$ test; $P < 0.001$) less (232 ± 7.2 g; mean ± S.E.M.; $n = 8$) than controls (381 ± 12.53 g, $n = 8$). Similarly, blood glucose level was much higher in diabetic rats (407.50 ± 35.90 mg dl$^{-1}$, $n = 8$, compared with control 81.20 ± 3.1 mg dl$^{-1}$, $n = 8$). Basal amylase secretion (expressed as U ml$^{-1}$ (100 mg tissue)$^{-1}$) by parotid glands from control and diabetic rats was 14.36 ± 0.89 ($n = 38$) and 2.36 ± 0.27 ($n = 38$), respectively. These results show that DM can elicit a significant ($P < 0.001$) decrease in amylase secretion compared with control. When control parotid segments were stimulated with either acetylcholine ($10^{-5}$ M ACh) or noradrenaline ($10^{-5}$ M NA), there was a marked increase in amylase secretion. Typically, amylase output in the presence of ACh and NA was 25.43 ± 1.47 ($n = 6$) and 64.10 ± 9.9 ($n = 9$), respectively. The output elicited by both ACh and NA from diabetic parotid segments was significantly ($P < 0.05$) reduced compared with that elicited from controls. Values with $10^{-5}$ M ACh and $10^{-5}$ M NA were 14.88 ± 2.49 ($n = 5$) and 36.60 ± 6.7 ($n = 4$), respectively. Basal $[Ca^{2+}]_i$ in fura-2-loaded acinar cells from control parotid glands was 93.10 ± 6.73 nM ($n = 8$). Stimulation of acinar cells with $10^{-5}$ M ACh resulted in no significant change in the initial peak $Ca^{2+}$ transient in diabetic compared with control acinar cells. In contrast, the plateau phase of the $Ca^{2+}$ transient was markedly decreased in diabetic acinar cells compared with controls, suggesting that DM may be associated with a decrease in $Ca^{2+}$ entry into parotid acinar cells. Light microscopic studies of diabetic and age-matched control parotid glands showed striking differences in morphology. Diabetic parotid glands contained a large number of lipid vacuoles, whereas glands from control animals displayed normal structure and with few or no lipid vacuoles. The results have shown that DM can lead to the infiltration of numerous lipid vacuoles and a reduction in amylase secretion (salivary insufficiency) in the rat parotid gland.

Francis, L.P. *et al.* (1990). *Exp. Physiol.* **75**, 669–680.

Sharma, A.K. *et al.* (1985). *J. Neurol. Sci.* **67**, 285–297.

Singh, J. *et al.* (1999). *Exp. Physiol.* **84**, 20–38.

*All procedures accord with current UK legislation.*

## Effects of extracellular magnesium on secretagogue-evoked secretory responses in the isolated rat submandibular gland

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In a previous study we have shown that magnesium ($Mg^{2+}$) can modulate secretagogue-evoked secretory responses in the isolated parotid gland (Yago *et al.* 1999). This study investigated the effects of perturbation of extracellular magnesium ($[Mg^{2+}]_o$) on secretagogue-induced secretory responses in the isolated rat submandibular gland. Donor rats were humanely killed by rapid cervical dislocation. The glands were isolated, cut into small segments (10–20 mg) and incubated in 20 ml of oxygenated Krebs-Henseleit solution for 30 min at 37 °C in a shaking water bath in the absence and presence of $10^{-5}$ M acetylcholine (ACh), $10^{-5}$ M noradrenaline (NA) or $10^{-5}$ M phenylepinephrine (PHE) during perturbation (0 mM, 1.1 mM, 5 mM, 10 mM) of $[Mg^{2+}]_o$. Following incubation, the tissues were removed, blotted dry and weighed. Total protein concentration in effluent samples was measured using a colorimetric method (Lowry *et al.* 1951) and expressed as $\mu g\ ml^{-1}$ (100 mg tissue)$^{-1}$; $n = 6$–8 except where stated. Submandibular acinar cells were isolated and loaded with fura-2 for the measurement of $[Ca^{2+}]_i$ using the cell suspension method (Lennard & Singh, 1992).

Basal protein outputs in zero, normal (1.1 mM) and elevated (5 mM and 10 mM) $[Mg^{2+}]_o$ were (means ± S.E.M.) 201.4 ± 11.7, 249.5 ± 13.3, 154.84 ± 11.3 and 175.1 ± 6.9, respectively. These results indicate that both zero and elevated $[Mg^{2+}]_o$ can reduce basal protein output compared with normal $[Mg^{2+}]_o$. Stimulation of segments with $10^{-5}$ M ACh, $10^{-5}$ M NA or $10^{-5}$ M PHE resulted in marked increases in total protein output in normal $[Mg^{2+}]_o$ compared with responses obtained in both zero and elevated $[Mg^{2+}]_o$. Protein outputs in response to ACh, NA and PHE respectively were, in zero $[Mg^{2+}]_o$, 203.7 ± 10.5, 320.9 ± 11.6 and 254.9 ± 8.9; in 1.1 mM $[Mg^{2+}]_o$, 303.4 ± 22.7, 491.8 ± 21.7 and 319.9 ± 12.91; in 5 mM $[Mg^{2+}]_o$, 205.1 ± 9.6, 216.7 ± 9 and 202.7 ± 10.1, and in in 10 mM $[Mg^{2+}]_o$, 209.7 ± 30.7, 253.2 ± 16.3 and 213.2 ± 15.5. Basal $[Ca^{2+}]_i$ in zero, 1.1, 5.0 and 10.0 mM $[Mg^{2+}]_o$ was 106.2 ± 8.6, 104.5 ± 5.6, 47.7 ± 2.8 and 57.8 ± 8.7 nM (all $n = 6$–20), respectively. Stimulation of acinar cells with $10^{-5}$ M ACh resulted in marked increases in both the initial peak and plateau phase of the $Ca^{2+}$ transient in normal $[Mg^{2+}]_o$. However, in zero and elevated $[Mg^{2+}]_o$ both the peak and the plateau phases of the ACh-induced $Ca^{2+}$ transient were significantly (ANOVA plus *post-hoc* tests; $P < 0.05$) reduced compared with the responses obtained in normal $[Mg^{2+}]_o$. The results indicate that $Mg^{2+}$ can influence secretagogue-evoked secretory responses in the submandibular gland possibly by controlling $Ca^{2+}$ mobilization.

Lennard, R. & Singh, J. (1992). *Gen. Pharmacol.* **23**, 903–908.

Lowry, O.H. *et al.* (1951). *J. Biol. Chem.* **193**, 265–275.

Yago, M.D. *et al.* (1999). *J. Physiol.* **520.P**, 22*P*.

*All procedures accord with current UK legislation.*

## Effects of ageing on the morphology and α-amylase release in the isolated rat parotid gland

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In a previous study we have shown that the rat lacrimal gland acinar granules change from serous to seromucous and subsequently to mucous in nature during ageing. Moreover, these changes are associated with decreased tear secretion in both quantity and composition (Draper *et al.* 1999). Since the parotid salivary gland is similar in structure and function to the lacrimal gland, then it is possible that the ageing process may also affect the structure and function of the parotid gland, which may lead to such complications as dry and burning mouth syndromes. The aim of this study was to investigate the morphology and α-amylase secretion in parotid glands obtained from young and ageing male Wistar rats. Four age groups of 2- to 3-month ($n = 4$), 12-month ($n = 4$), 16-month ($n = 4$) and 20-month-old ($n = 4$) rats were humanely killed and used for the morphological study using established methods (Draper *et al.* 1999). Acini counts were performed using light microscopy at ×40 magnification and a total of ten random fields were taken from four slides using four animals for each age group. For α-amylase measurement, acini were isolated from 2- to 6-month and 12-month-old parotid glands, and incubated for 30 min at 37 °C in a shaking water bath with different concentrations of acetylcholine (ACh, $10^{-9}$ M to $10^{-4}$ M), noradrenaline (NA, $10^{-9}$ M to $10^{-4}$ M), or isoprenaline (ISOP, $10^{-9}$ M to $10^{-4}$ M). α-Amylase release was measured using the Phadebas blue starch method (Ceska *et al.* 1969).

Light microscopic investigations into age-related changes in the isolated rat parotid gland showed marked differences in the organisation and distribution of parotid acini within the gland. Typically, in young (2–3 months as controls) parotid glands, the acini were visible in a compact arrangement, with defined parotid ducts, and with little or no connective tissue and lipid infiltration. At 12 months no striking age-related morphological change was noticed compared with 2- to 3-month-old glands. In contrast, glands from 16- and 20-month-old animals showed a spacious acini distribution, and enlarged parotid ducts with numerous and thick connective tissue septa which were localised around and within the parotid ducts. Lipid accumulation was also apparent in 16- and 20-month-old glands when compared with control. Acini counts indicated a significant (Student's unpaired *t* test; $P < 0.01$) decrease in the mean acini distribution, when compared with control mean acini count (mean ± S.E.M.; 286 ± 16) ($n = 4$). Acini counts for 12, 16 and 20 months were 208 ± 8.08 ($n = 4$), 167.77 ± 7.82 ($n = 4$) and 138 ± 11.4 ($n = 4$), respectively. Basal amylase release from parotid acini of 2- to 6-month and 12-month-old glands was 6.76 ± 1.06 ($n = 8$) and 4.98 ± 0.88 % of total ($n = 8$), respectively. Incubation of acini from 2- to 6-month-old gland ($n = 8$) with ACh, NA or ISOP (all $10^{-9}$–$10^{-4}$ M) resulted in marked dose dependent increases in amylase release with maximal effect occurring at $10^{-5}$ M. In contrast, parotid acini from 12-month-old glands released significantly ($P < 0.05$) less amylase during stimulation with different concentrations of ACh, NA or ISOP ($n = 8$ for each). The results indicate that ageing can lead to marked morphological changes and a decrease in amylase release from the rat parotid gland.

Ceska, M. *et al.* (1969). *Clin. Chim. Acta* **26**, 437–444.

Draper, C.E. *et al.* (1999). *Exp. Eye Res.* **68**, 265–276.

*All procedures accord with current UK legislation.*

Putney, J.W. Jr (1986). *Cell Calcium* **7**, 1–2.

*All procedures accord with current national and local guidelines.*

## Extracellular magnesium regulates secretagogue-evoked calcium mobilisation in rat parotid acinar cells

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Calcium ($Ca^{2+}$) plays an important physiological role in mediating salivary gland secretion (Ambudkar, 2000). Cytosolic $Ca^{2+}$ is derived from two sources, intracellular stores such as the endoplasmic reticulum and from extracellular medium. Secretagogue-evoked $Ca^{2+}$ influx is referred to as capacitative $Ca^{2+}$ entry (CCE) since it is the driving force which is responsible for prolonged and sustained salivary secretion (Putney, 1986). The precise mechanism involved in the regulation of CCE is still not fully understood (Ambudkar, 2000). This study investigated the effect of perturbation of extracellular magnesium $[Mg^{2+}]_o$ on CCE in isolated rat parotid acinar cells. Donor rats were humanely killed by rapid cervical dislocation, and parotid glands were surgically removed. An established method was used to prepare parotid acinar cells and load them with fura-2 (Baum *et al.* 1990). $[Ca^{2+}]_i$ was measured in single cells using microspectrofluorimetry (Lajas *et al.* 2000) in the absence and presence of $10^{-5}$ M ACh during perturbation of $[Mg^{2+}]_o$ (0, 1.1, 5 and 10 mM). Initially, cells were perfused with a $Ca^{2+}$ free medium containing the different $[Mg^{2+}]_o$ and 1 mM EGTA and then stimulated with ACh. After peak recovery, cells were perfused with a medium containing 2 mM $Ca^{2+}$ in different $[Mg^{2+}]_o$. $[Ca^{2+}]_i$ values in 35–40 experiments are expressed as mean (± S.E.M.) 340/380 ratio. All experiments were undertaken at room temperature.

Basal $[Ca^{2+}]_i$ in zero, normal (1.1 mM) and elevated (5 mM and 10 mM) $[Mg^{2+}]_o$ were 0.252 ± 0.006, 0.227 ± 0.009, 0.209 ± 0.013 and 0.211 ± 0.009 ($n$ = 35–40), respectively. Stimulation of fura-2-loaded acinar cells with $10^{-5}$ M ACh resulted in significant (ANOVA plus *post-hoc* test; $P < 0.05$) increases in the initial peak $Ca^{2+}$ transient ($Ca^{2+}$ released from intracellular stores) in zero and normal $[Mg^{2+}]_o$ compared with the respective controls. In contrast, elevated (5 mM and 10 mM) $[Mg^{2+}]_o$ attenuated the ACh-evoked $Ca^{2+}$ transient compared with the responses obtained in zero and normal (1.1 mM) $[Mg^{2+}]_o$. Typically, $[Ca^{2+}]_i$ in zero, normal and elevated $[Mg^{2+}]_o$ were 0.562 ± 0.016, 0.511 ± 0.016, 0.310 ± 0.018 and 0.293 ± 0.021 ($n$ = 35–40), respectively. Perfusion of fura-2-loaded acinar cells with a physiological salt solution containing 2 mM $Ca^{2+}$ resulted in significant ($P < 0.05$) increases in $Ca^{2+}$ uptake (CCE) in zero and normal $[Mg^{2+}]_o$ compared with their respective basal values. The peak CCE was much more rapid (35 ± 5 s) in normal $[Mg^{2+}]_o$ compared with a slower uptake (226 ± 6 s) in zero $[Mg^{2+}]_o$. In both 5 mM and 10 mM $[Mg^{2+}]_o$ CCE was significantly ($P < 0.05$) reduced and slower compared with the response obtained in normal (1.1 mM) $[Mg^{2+}]_o$. The results indicate that $Mg^{2+}$ can regulate secretagogue-evoked $Ca^{2+}$ mobilization (both its release from intracellular stores and CCE) in parotid acinar cells.

Ambudkar, I.S. (2000). *Crit. Rev. Oral Biol. Med.* **11**, 4–25.

Baum, B.J. *et al.* (1990). *Meth. Enzymol.* **192**, 26–37.

Lajas, A. *et al.* (2000). *Mol. Cell. Biochem.* **205**, 163–169.

## Morphological changes and secretory responses in streptozotocin-induced diabetic rat pancreas

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Very little is known about the morphology and secretory responses of the diabetic exocrine pancreas. In this study the morphology and effects of cholecystokinin octapeptide (CCK-8) on pancreatic juice flow and on $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ were investigated in the diabetic rat pancreas and compared with healthy controls.

Animals were rendered diabetic by a single injection of streptozotocin (60 mg $kg^{-1}$ I.P.; Sharma *et al.* 1985). Age-matched controls received a similar volume of citrate buffer. Seven weeks later, animals were either anaesthetized with urethane (1 g $kg^{-1}$ I.P.) for measurement of pancreatic juice flow or humanely killed and the pancreas isolated for $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ determinations using established methods (Lennard & Singh, 1992). After the *in vivo* experiments, rats were humanely killed by urethane overdose. Body weights in control rats initially (9 weeks old) and prior to experimentation (16 weeks old) were 268.0 ± 18.0 g (mean ± S.E.M., $n$ = 68) and 391.0 ± 31 g ($n$ = 10), and in diabetic rats were 250.3 ± 6.1 g ($n$ = 20) and 192.7 ± 4.5 g ($n$ = 10), respectively. Non-fasting blood glucose levels in control and diabetic rats were 92.40 ± 2.42 mg $dl^{-1}$ ($n$ = 8) and > 500 mg $dl^{-1}$ ($n$ = 10), respectively. This shows that diabetic animals gained significantly (Student's unpaired $t$ test; $P < 0.05$) less weight and had higher glucose levels than controls. There were also marked morphological differences in the number and pattern of distribution of insulin-, glucagon- and somatostatin-immunoreactive cells. At the light microscope level the morphology of acinar cells of diabetic rats was similar to that of non-diabetics. However, ultrastructural studies showed empty vacuoles in pancreatic acinar cells of diabetic rats, whereas they were not observed in control animals.

Resting (basal) pancreatic juice flow in control and diabetic anaesthetized rats was 0.49 ± 0.04 $\mu$l $min^{-1}$ ($n$ = 10) and 1.17 ± 0.09 $\mu$l $min^{-1}$ ($n$ = 11), respectively, indicating that diabetes results in a significant ($P < 0.05$, Student's unpaired $t$ test) elevation in basal flow rate. Continuous infusion of CCK-8 (150 pmol $kg^{-1}$ $h^{-1}$) resulted in a significant ($P < 0.05$, one-way ANOVA) increase in flow rate in control animals, peaking within 20 min (1.28 ± 0.19 $\mu$l $min^{-1}$, $n$ = 5). In diabetic rats, flow rate increased moderately, reaching a maximum after 60 min (1.54 ± 0.22 $\mu$l $min^{-1}$, $n$ = 8). Basal $[Ca^{2+}]_i$ in control and diabetic fura-2-loaded acinar cells was 109.40 ± 15.41 nM ($n$ = 15) and 130.62 ± 17.66 nM ($n$ = 8), respectively. CCK-8 ($10^{-9}$ M) induced a peak response of 436.55 ± 36.54 nM ($n$ = 15) and 409.31 ± 34.64 nM ($n$ = 8) in control and diabetic cells. Basal $[Mg^{2+}]_i$ in control and diabetic mag-fura-2-loaded cells was 1.00 ± 0.06 mM ($n$ = 18) and 0.88 ± 0.04 mM ($n$ = 10), respectively, and stimulation with $10^{-8}$ M CCK-8 evoked a slow decrease in both groups to reach a new steady level (0.80 ± 0.05 mM ($n$ = 18) and 0.60 ± 0.02 mM ($n$ = 10) in controls and diabetics, respectively). At this point $[Mg^{2+}]_i$ in

diabetic acinar cells was significantly ($P < 0.05$, Student's unpaired $t$ test) lower compared with control cells.

The results indicate that diabetes is associated with an increase in basal pancreatic juice flow and a decrease in the secretory response to CCK-8 compared with control.

Lennard, R. & Singh, J. (1992). *Gen. Pharmacol.* **23**, 903–908.
Sharma, A.K. *et al.* (1985). *J. Neurol. Sci.* **67**, 285–297.

*All procedures accord with current UK legislation.*

## Transthyretin (TTR) inhibits the efflux of thyroxine (T4) from the cerebrospinal fluid and increases the uptake into brain

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The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are synthesized outside the brain, and are essential for normal growth and development of the central nervous system (Moore *et al.* 1973; Pickard *et al.* 1987). T4 can enter the brain via two pathways, directly through the blood–brain barrier (BBB) and across the choroid plexus (CP) into the cerebrospinal fluid (CSF) and then diffusing into the extracellular space around the neurones. TTR is a thyroxine-binding protein, synthesized by the CP and secreted into the CSF, and is proposed to play a role in the transport of T4 in the brain. However, the role of TTR in the distribution of T4 into different areas of the brain is still controversial. By using the bilateral ventriculo-cisternal perfusion (VC) technique (Davson & Segal, 1970) in anaesthetized rabbits (Domitor and pentobarbitone given I.V. at a dose of 0.5 mg and 10 mg $kg^{-1}$, respectively), we have investigated the efflux of $^{125}$I-T4 (10 $\mu$Ci (50 ml)$^{-1}$) out of the ACSF perfusate, and the uptake into the surrounding tissues (animals were killed humanely at the end of the experiment). We have shown a large loss of T4 from the CSF, about 45 %. At a TTR concentration of 2.5 $\mu$g $ml^{-1}$ ACSF, as TTR/T4 complex, the loss was significantly inhibited by 25 % ($P = 0.004$, Student's unpaired $t$ test, $n = 3$). $^{125}$I-T4 uptake into the brain was significantly increased in the presence of TTR as TTR/T4 complex, i.e. caudate nucleus uptake increased from 8.23 ± 2.87 to 45.11 ± 8.03 ml (100 g)$^{-1}$ ($P = 0.006$, $n = 3$); values are means ± S.E.M. We conclude that TTR increases the uptake of T4 into the surrounding brain tissues and decreases the loss from the CSF. These results suggest that TTR plays an important role in the removal and distribution of T4 in the central nervous system. Studies are in progress to determine the route and possible mechanism of these processes.

Davson, H. & Segal, M.B. (1970). *J. Physiol.* **209**, 131–153.
Moore, T.J. *et al.* (1973). *Am. J. Physiol.* **225**, 925–929.
Pickard, R. *et al.* (1987). *Endocrinology* **121**, 2018–2026.

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## Effect of stress and circadian variations of phagocytosis and anion superoxide levels in macrophages

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Circadian variations have been observed in several immune system functions, allowing the immune cells to be active and ready at specific times of the day for greater effectiveness. The daily and seasonal rhythms of immune functions are regulated and co-ordinated by the neuroendocrine homeostatic system. In the present work we evaluated the circadian rhythm of phagocytosis and superoxide anion levels in macrophages in control and stress situations. Male Wistar rats (*Rattus norvegicus*), 3 months old, were maintained under a 12 h light and 12 h dark photoperiod. The stressor was forced swimming to exhaustion, with the rats subjected to free swimming for 2 h and 15 final minutes swimming on a rotating rod test that is a modified version of the automated Porsolt test (Thornton *et al.* 1990). The animals were subjected to this stress every 2 h over one circadian period. After killing the animals by decapitation, the macrophages were obtained from the peritoneal cavity. The functional capacity of macrophages was evaluated by the phagocytosis index (PI, number of latex beads ingested by 100 macrophages) and the superoxide anion levels by a quantitative nitroblue tetrazolium test (Rodriguez *et al.* 2001). The rats were handled according to the guidelines of the European Community Council Directive 86/6091 EEC. All data are expressed as mean values ± S.D. Data were compared by Student's unpaired $t$ test. Values of $P < 0.05$ were considered significant.

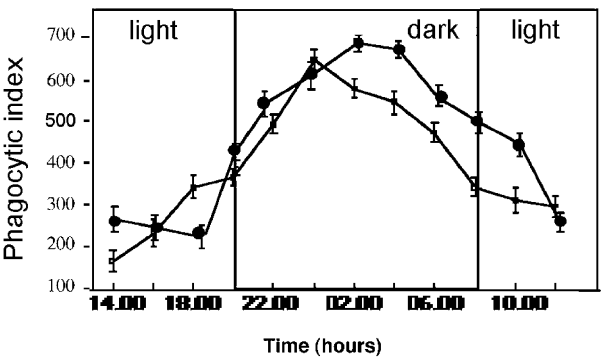


Figure 1. Plot of the phagocytosis index presented by peritoneal macrophages from control and stressed rats. □, control; ●, stress. Each value represents the mean ± S.D. of six determinations.

The results (Fig. 1) showed that in the control rats, the phagocytic activity is higher during darkness with maximum values at 24.00 h (639 ± 25 PI, $n = 6$), and declines during daylight to minimum values at 14.00 h (153 ± 34 PI). For the stressed animals, there were also day/night variations in the phagocytic activity, but now the dark-period values were higher than those of the basals, with the maximum values being obtained at 02.00 h (698 ± 30 PI). For the superoxide anion levels (Fig. 2) produced in the macrophages during oxidative metabolism, the cells from the control animals presented a rise during the daylight period, with maximum levels reached at 10.00 h. The stress led to a loss of the circadian rhythm in superoxide anion levels, being raised in the stressed animals in a general fashion over the length of the circadian cycle relative to those found in the control situations.

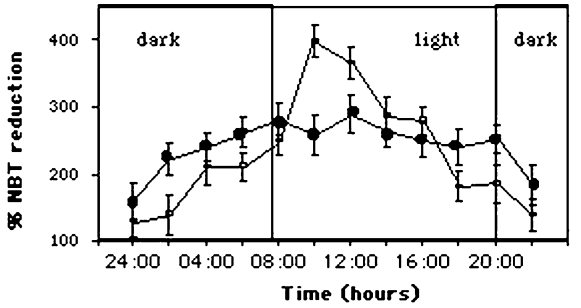


Figure 2. Plot of NBT reduction presented by peritoneal macrophages from control and stressed rats. □, control; ●, stress. Each value represents the mean ± S.D. of six determinations

In conclusion, these data suggest that stress leads to alterations in the non-specific immune response.

Rodriguez, A.B. *et al.* (2001). *J. Pineal Res.* **31**, 31–38.

Thornton, E.W. *et al.* (1990). *Behav. Neurosci.* **104**, 37–43.

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*All procedures accord with current national guidelines.*

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## Surfactant/water systems as models for the effects of anaesthetics on biological membranes

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Concentrated solutions of surfactant/water systems exhibit phases that can be used as models for biological membranes. Whilst such phases will lack the molecular complexity of real membrane systems, they provide simplified systems within which it is possible to elucidate the action of biologically active compounds where an obvious membrane interaction is not readily identifiable, e.g. inhalation anaesthetics. Anaesthetics have clear actions on the biophysical properties in both cardiac myocytes and nerve cell bodies. One possible explanation for their action is that the anaesthetic molecules reside in the interfacial region of the membranes promoting change of the interfacial curvature, possibly resulting in the formation of intra-membrane pores. In surfactant systems the role of interfacial curvature controls the formation of any lyotropic liquid crystalline phase and its resultant stability. The sensitivity of such phases to alteration in interfacial packing makes them ideal systems to monitor the effect of solubilised anaesthetics, if they are concentrated in this region.

Here, the stability and structural evolution of a number of model membrane systems formed by commercially available surfactants has been studied as a function of anaesthetic type and concentration. The mole ratio of the anaesthetic in the samples was in the range of 0.0010–0.0080 for surfactant mole ratios in the range of 0.0145–0.0355. Experiments were run in the temperature range 20–60 °C using a general inhalation anaesthetic, halothane ($n = 3$), and a local anaesthetic, lidocaine ($n = 2$). The results indicate a general trend consisting of dehydration of the interface coupled with a preference for the formation of phases that possess lower rather than higher interfacial curvature, with the latter an observation which precludes the formation of porous lamellae. However, recent NMR and optical microscopy indicate that the stabilisation of higher curvature may be possible for a limited number of anaesthetics.